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Award Number: DAMD17-00-1-0349

TITLE: Prevention of Breast Cancer by IGFBP

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REPORT DATE: June 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 2002	Annual (1 Jun 01 - 31 May 02)

4. TITLE AND SUBTITLE
Prevention of Breast Cancer by IGFBP

5. FUNDING NUMBERS
DAMD17-00-1-0349

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*
In this proposal, we hypothesize that inhibition of IGF action by IGFBP-1 will prevent breast cancer in a SV40 Tag transgenic model of breast cancer. We will test this hypothesis with two specific aims: 1) to inhibit IGF action at the mammary epithelial cell by creating transgenic mice that express IGFBP-1 under the control of the whey acidic protein (WAP) promoter and 2) to test the ability of IGFBP-1 to suppress tumorigenesis by mating these animals with C3/Tag transgenic mice. To date, we have generated two founder lines containing the IGFBP-1 transgene and several F1 and F2 animals were analyzed. Unfortunately, while the transgene was clearly integrated into these animals, we were unable to detect expression of IGFBP-1 protein. To correct this problem we have generated more founders with a modified construct involving insulator sequences. Oocytes have been injected and we are awaiting the offspring.

14. SUBJECT TERMS
breast cancer

15. NUMBER OF PAGES
4

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Introduction
The purpose of this project was to create transgenic mice expressing IGFBP-1 in the mammary gland. We hope overexpression of this binding protein can neutralize IGF action and inhibit breast cancer development.

Body
To create these animals, we cloned a IGFBP-1 cDNA into a whey acidic protein promoter construct. Embryos were injected into animals and founders were created. Of the animals we have analyzed, approximately 25% have the transgene. From this founder line, we have selected two separate lines and mated them with wild-type animals. As shown below, while the transgene was present in many of the offspring. However, uwe were unable to identify IGFBP-1 protein in the offspring.

Key Research Accomplishments
Two founder mice were selected for further study. Figure 1 shows the results of a southern blot of a founder female mouse and several offspring mated to a wild-type male mouse. As can be seen, this founder female (F2683) has integrated the transgene and several of the offspring of the mating also contain the transgene.

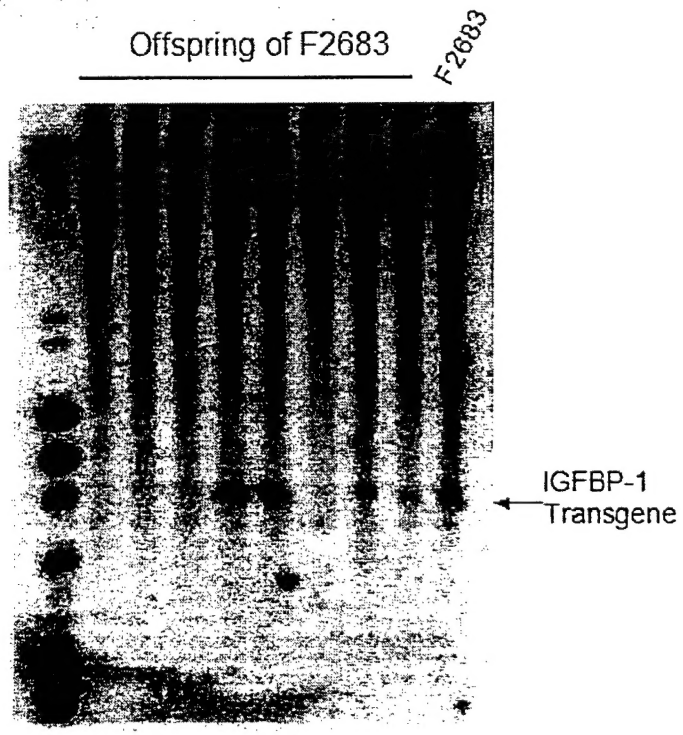

Figure 1 - Southern blot of IGFBP-1 in founders and offspring Genomic DNA was isolated from tails and

We then mated the offspring and collected milk and dissected the mammary glands after a pregnancy. We analyzed milk protein and extracted protein from the mammary gland. We used two methods to screen for IGFBP-1 expression. Immunoblot using a human specific antibody and ligand blot using radiolabelled IGF-I to detect IGF binding protein species were used on over 30 separate mammary glands from different offspring. We initially had positive results suggesting that IGFBP-1 protein could be detected by immunoblot in some of the offspring. However, there were conflicting results with ligand blot, as animals with positive IGFBP-1 immunoblot seemed to have negative IGFBP-1 when detected by ligand blot (data not shown). Since it was possible that the IGFBP-1 antibody was detecting a non-specific protein, e also isolated mRNA from the dissected mammary gland. Using immunoblot, ligand blot, and ribonuclease protection assay, we were unable to detected IGFBP-1 mRNA.

While were disappointed by these findings, there are several potential explanations for this finding. First, although the transgene was integrated, the expression may not be stable. It is possible that expression of the transgene was selected against over time or had a deleterious effect on lactation. It is possible that transgene expression was selected against during mammary gland development. If this is the explanation, then it may be very difficult to express high levels of IGFBP-1 in the mammary gland. Second, selection of additional founders may enhance the ability to detect the protein. Hopefully, this is the reason for our inability to detect IGFBP-1 and if we screen additional animals, we will be able to select IGFBP-1 expressors.

Reportable outcomes
While transgene integration was documented, protein was not identified.

Conclusions
Additional animals need to be created. We have re-engineered the WAP-IGFBP-1 construct to contain insulator sequences. If the gene is silenced, then we will hopefully minimize this with silencer sequences. We have started a second round of animal selection, we will examine more animals with the construct we previously made and with the WAP-IGFBP-1 construct with insulator sequences.

References – None

Appendices - None